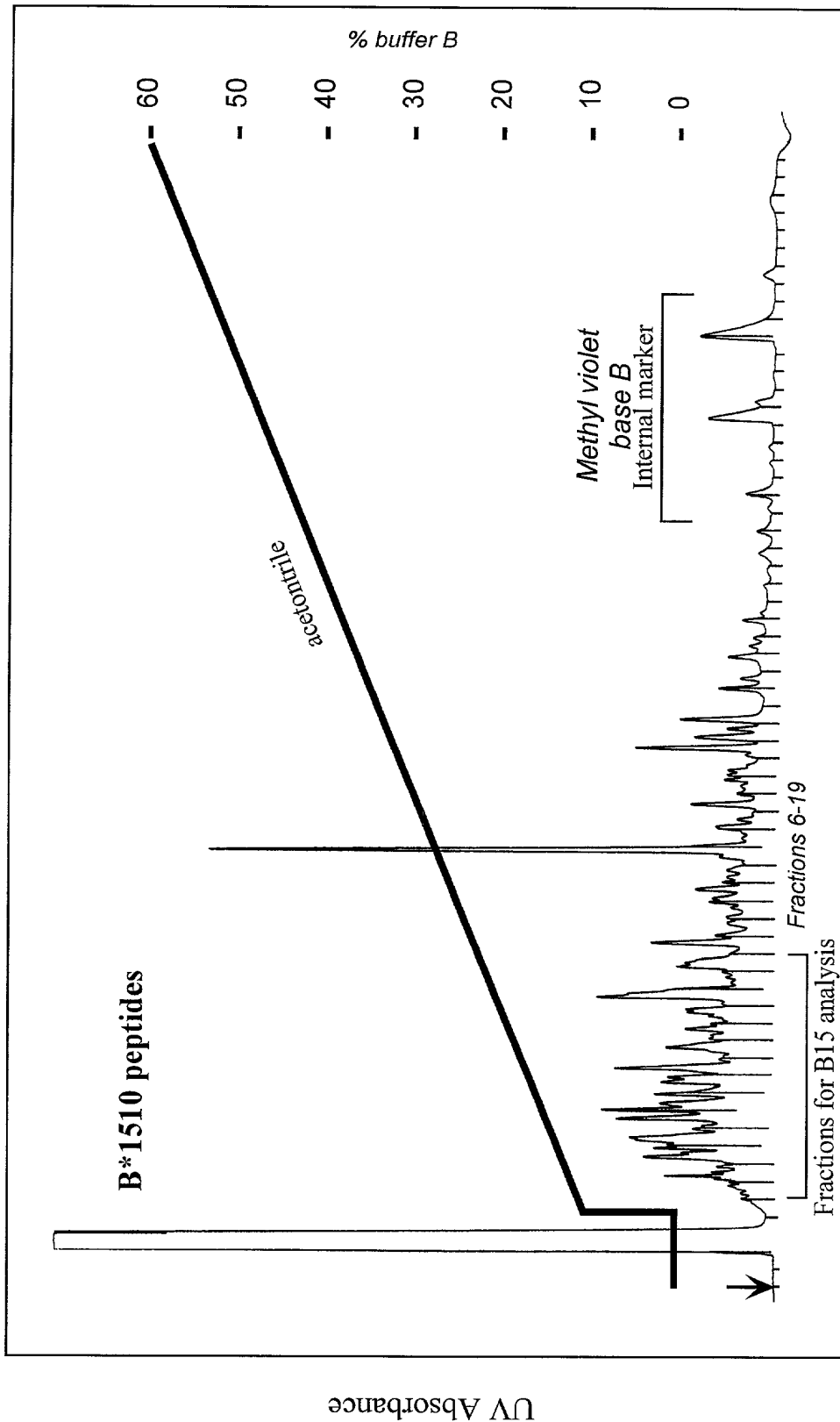


# Reverse phase HPLC of class I HLA eluted peptide ligands



Fraction Number

Fig. 1

Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

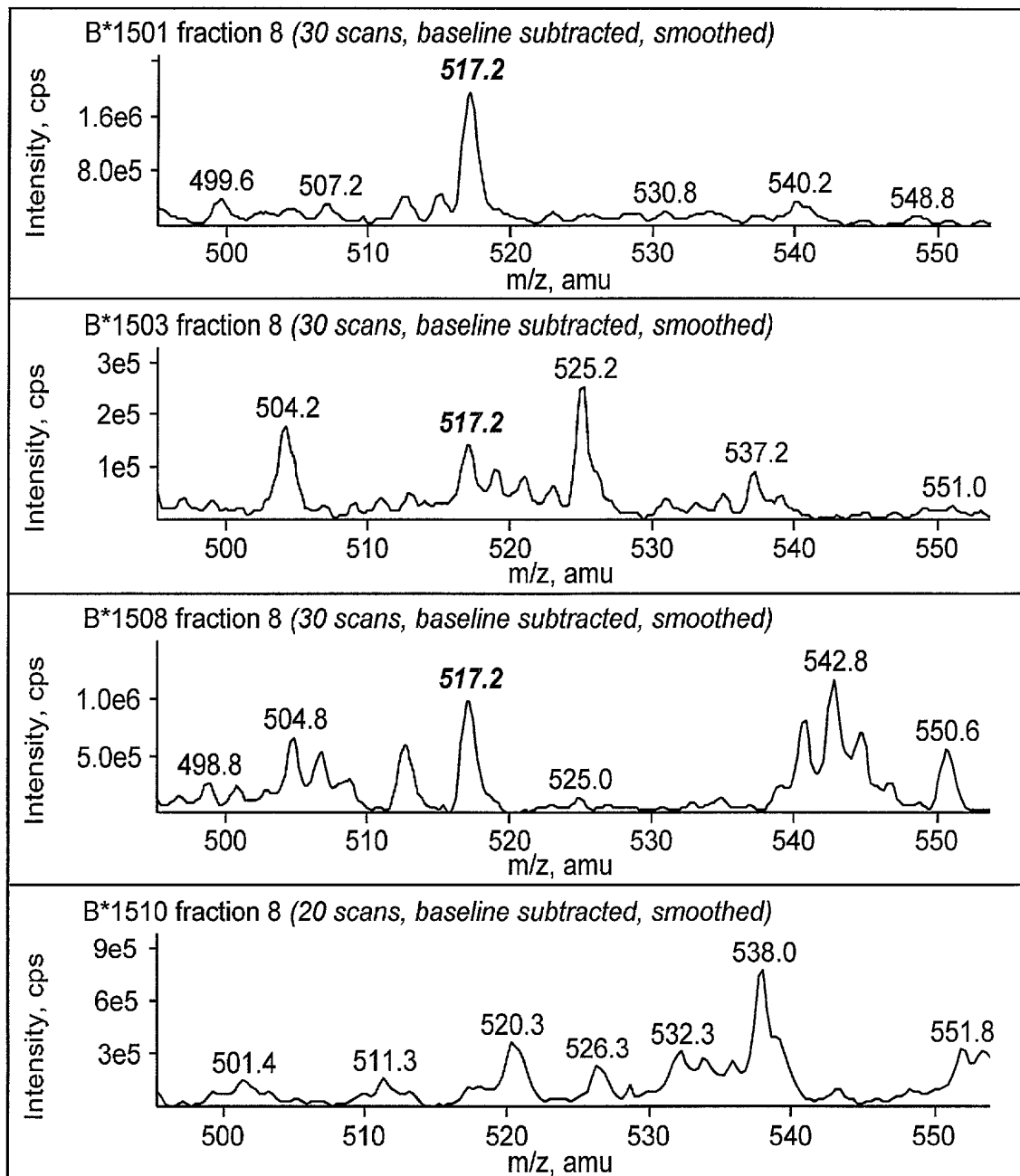


Fig. 2

MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I sHLA molecules. This data was accomplished by completing a second nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how ions can be MS ion mapped and subsequently MS/MS sequenced. There is sufficient peptide present to do multiple MS/MS fragmentation runs. There is also sufficient peptide present to facilitate a submotif on fraction 8 or further separation in the event that two peptides had mapped at 517.2 in the ion map.

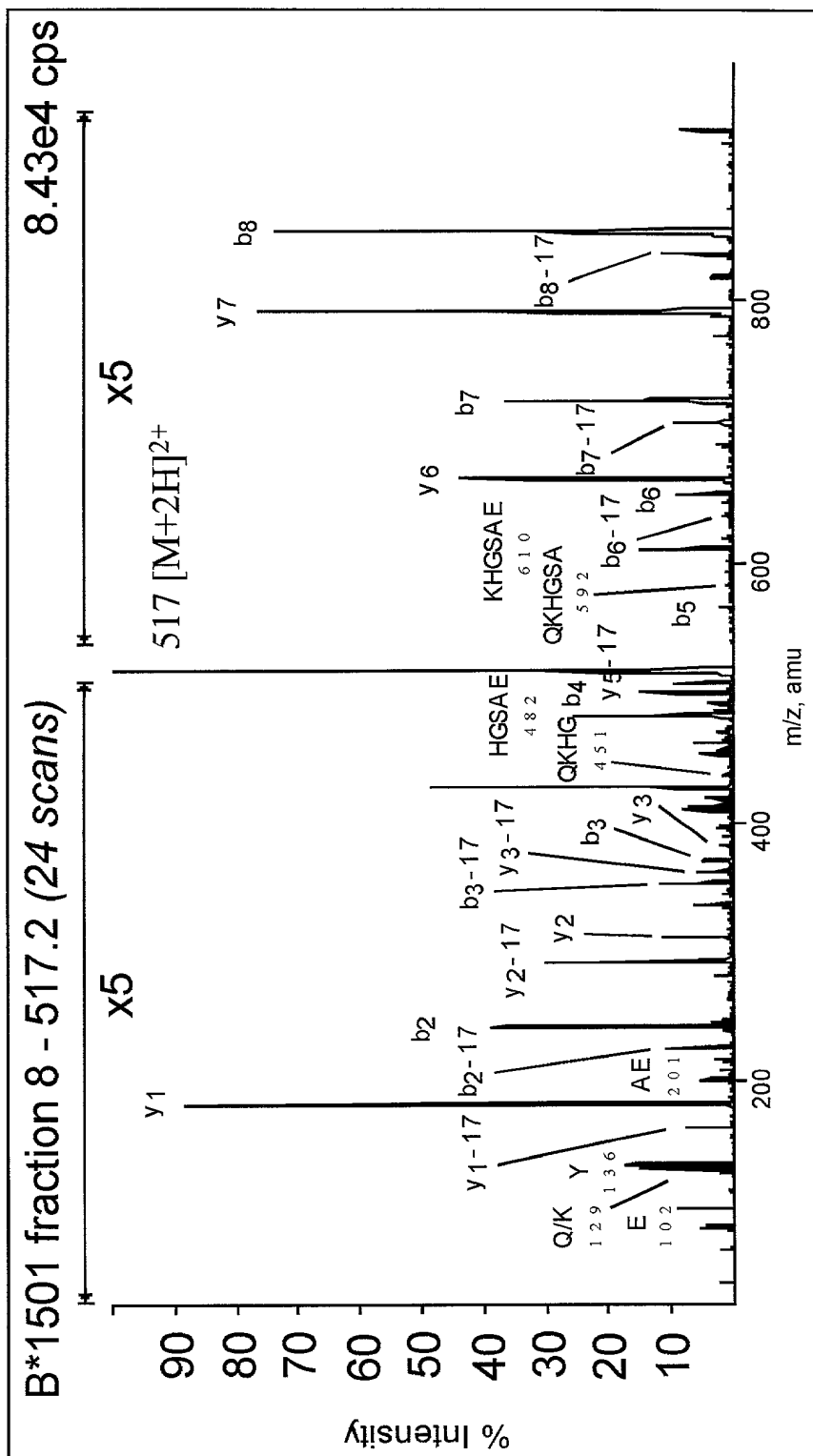


Fig. 3 1 of 3

Serial No. 10/082,034 Dkt. No. 6680.040  
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AND USING SAME  
Applicant: William Hildebrand Group No.: 2171  
Filed: 02/21/2002 Examiner: Unknown  
Atty: Douglas J. Sorocco Tel: (405) 478-5344

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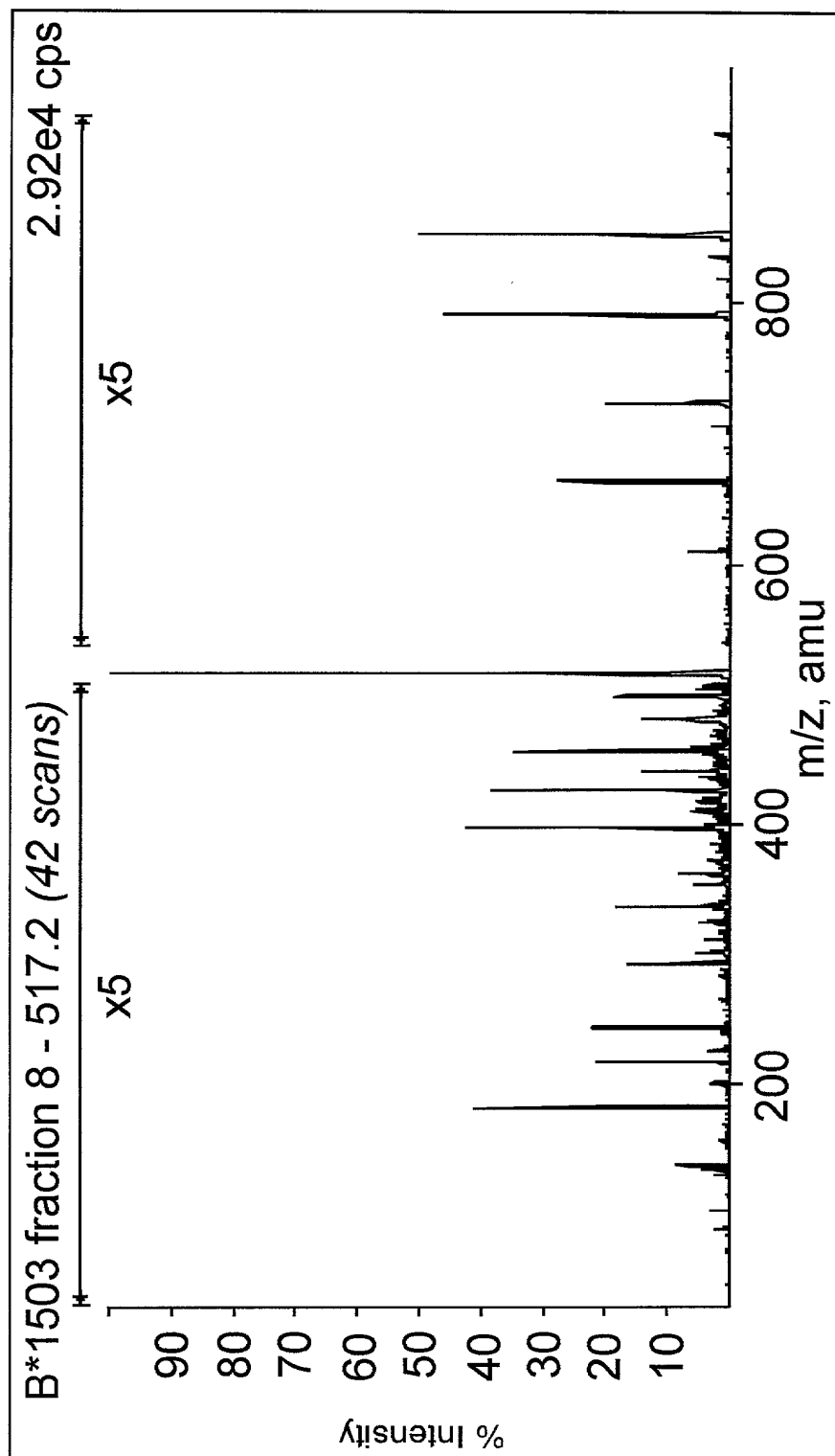


Fig. 3 2 of 3

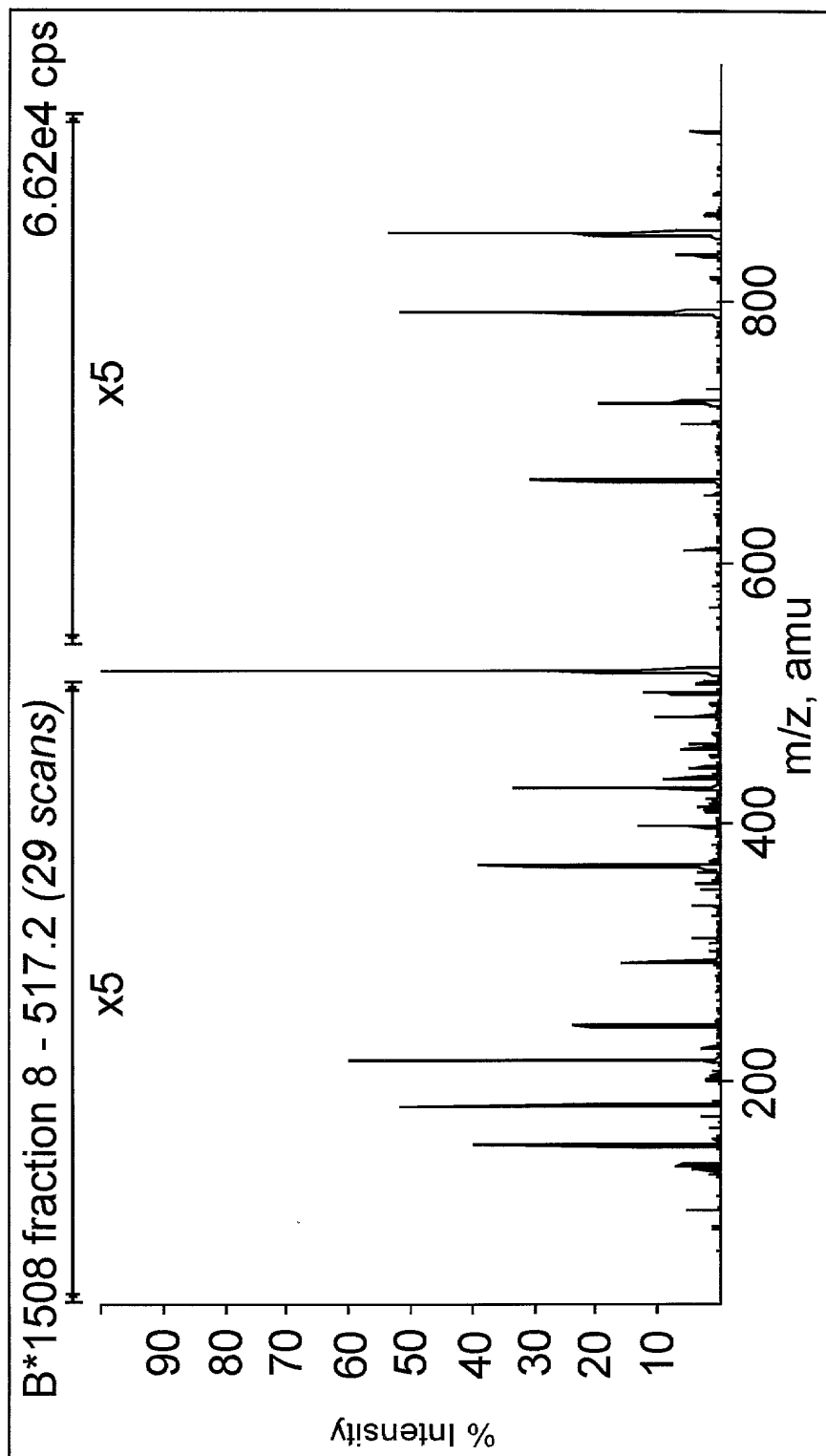
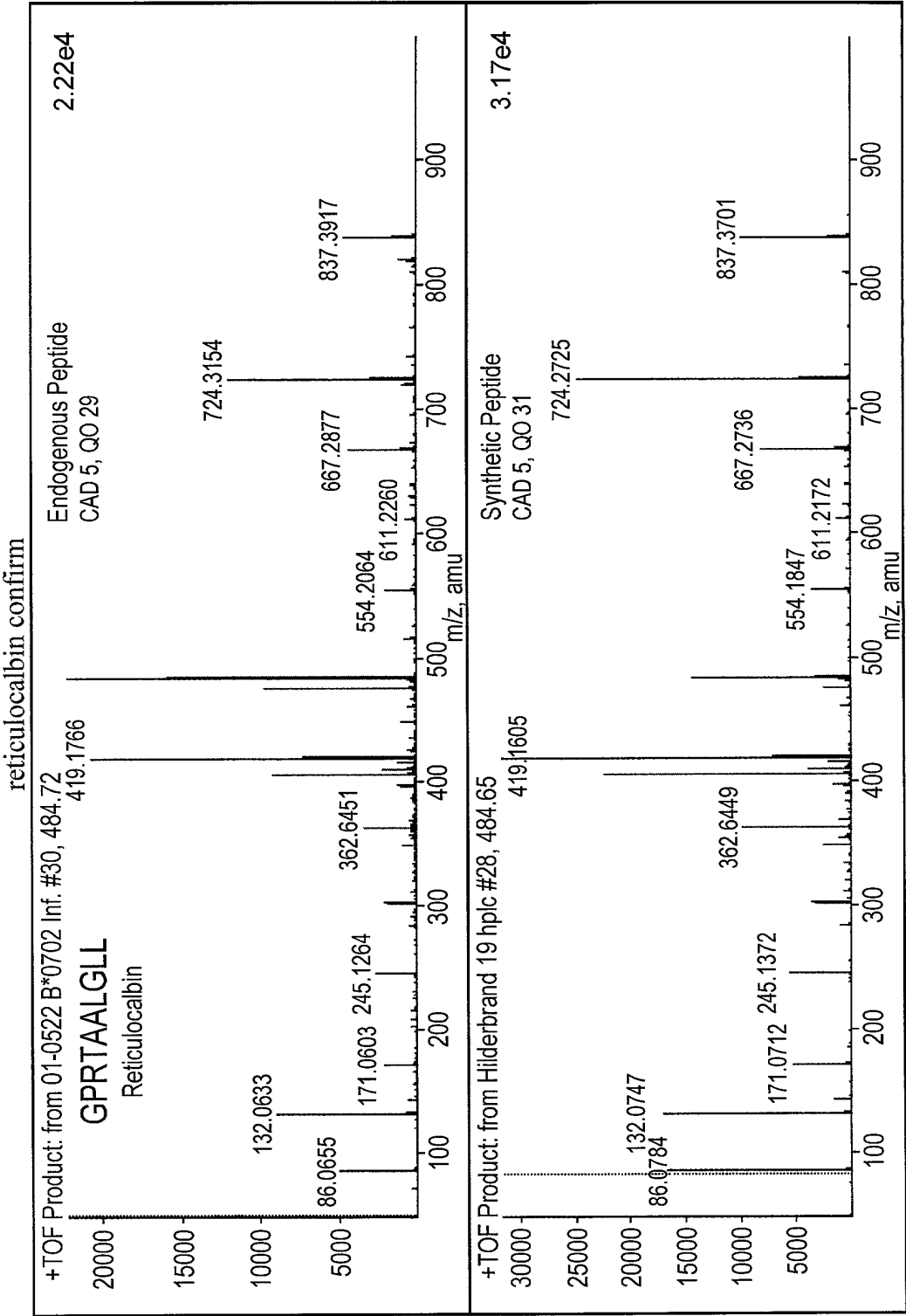


Fig. 3 3 of 3



sHLA B\*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B\*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

Fig. 4

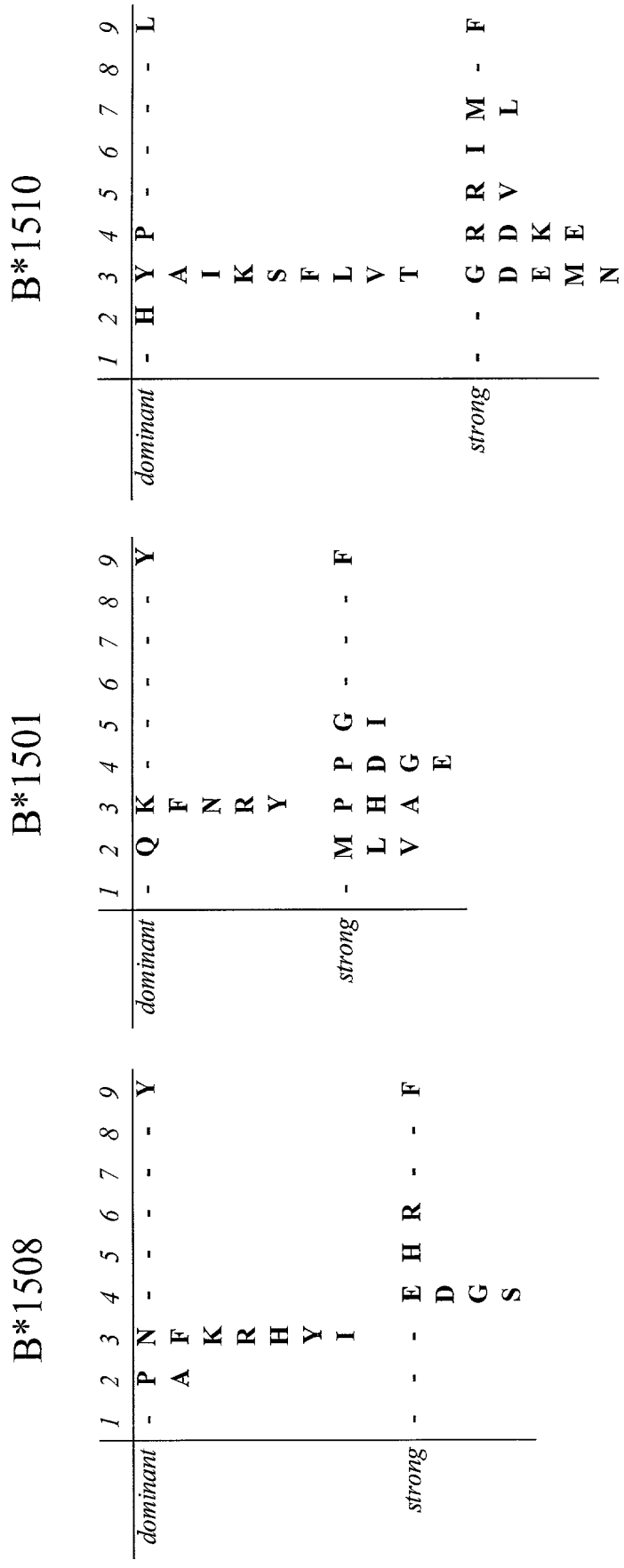


Fig. 5

--WDRHXTF	AQYAAAGESFY	ILGPPGGSVY	--WDRHXTF	FLZAMGSTY	YMWV---F	SVSXPHAP
TPRTHZHEY	YM---FMY	XLGDVNMY	----YT	GOYVZPTY	APAV---VGY	APFTGGNGX
XZYYEHTY	MVGXHPAT	AP---FVSY	ALGA---RGY	PMFDPPZTF	---TGF	EHVASSPAL
NGXAMHWTY	YMSGXYGTF	YMSGXYGTF	XS---VEY	XAVGHSGGT	PVPNVRXNY	HHAPCGVSX
VPCGZGSY	DPHYVSGHZF	---AVVAZSY	AQFASGAGZ	----PTY	---TXSX	NHAIIVSTSV
TAZKHRYG	VWACV---Y	MPAGYNNVY	-G-CDY	----PSY	YMWCAAEY	GHNZSVTSV
NQZHGSAEY	PLA-N-HTY	XPVVPAAZTY	---ZARGY	EPAMVXZCF	---XRDXY	SHZAPCTSV
TP---AY	VVAPITITGY	YXRYRMV	ALNGRVTMY	XAHTCEPRGY	SLX-----F	FVARFVSX
-P--GZDEV	XAXYRMV	YMDPSGVS	DHPAPPY	VOGPVGZVY	ALGSZAXMPF	HHSDBGSVSL
NGYDGNAGY	PXAMQXYTY		XAZVZMTAY	TGAPVSEEGY	VGYVDDTQF	MCZ-GMPAX
TPXGEPIYZSY	-P-MFGXAY		NQZHGSAEY	VOXYGYSV	DVEGWMSZY	XHSZPAGPAX
FVSNHAY	---TZNAY		FGXACXATSY	GQGPAGXGZY	QAHPXSARF	XHVVS--VX
SQFGGGSQY	MAAMVGVAVY		APMARGZY	GPPHNGXRAY	-----ZAY	YHGSZNPX
SQFDHVTY	LPHQPLATY		TG-----AY	AAHWHVEAY	GLGZTSAEF	EHGXENGH
XAN--VT	FVTXNXYEY		GOZZAVDF	TPPTRRESY	NAXG--RESSE	AHZAPOPXTX
VDX---Y	GPZVMZHGY		TPXGEPIYZSY	FPTDRRSZF	TARVXSVEY	FTACZNPAX
CPLSCFT	FQARXTEY		GQHASVXS	YTGVSYXHF	AAFCG---XV	SHAGAGXVX
FLZAMZSTY	AAAXV---VTY		FVSNHAY	AOASAPDAY	XLH--ET	GHXEGPXX
TVXDSZTHY	XPEMGZFSY		---SY	GQRKGAGSVF	ILGPPGSVY	XHGGDHVX
DPSGIZSGF	YV--VR-VF		NPPAZZPN	VOYXPF	XLGDVNMY	YHHDXXVX
---PGFY	AAVPGAXESY		-Q-DPPDMZY	ATGTAZNXNZY	VMGXTNANF	-VFSVHTTF
-A-PHPMGY	YVA---PAF		XQ--AGGZY	VWACV---Y	AVVTXZSDF	FH---XXX
AQTVGYGEY	VGY--AHPGF		SQFGGGSQY	PLA-N-HTY	AMNPTNTVF	EH---TVX
---SVY	---STY		SQFDHVTY	VWAPITTY	SQXAAGVDVF	MAX---VV
TGNCSGTGTY	SPTYTHAVAF		---AAHVPPGY	XQYIVGYF	---FVSVHTTF	---PVX
AQVNPSXTY	MPTA--MVMAF		FMDVGAPTIV	PLFGZTAGZY	XQGHHEMFY	XHYDRNZX
TP--ARAPT	XA---SYTY		XAN--VT	A-----ZXEY	PQZMA--Y	---AXSV
SPGAETRAKY	VGYVDDTQF		AQM--SEY	VAGW-----F	HLTGNEATSF	XHWPVNEX
YXX---RTF	ZATNSVTSTY		CPLSCFT	SGAXDRAYZF	YMDPSGVS	-H-----PVF
YXXG---GAF	YATAGEMMAF		FLZAMZSTY	VOGPVGTDF		XHEVZPXHX
-P---PSSGY	TARVXSVEY		TVXDSZTHY	FQARXTEY		-HCGPGMPX
TP--GRMY	MPAADYEVAF		AQAAPFAGY	XAGFFXKEY		ETPEHAPVX
PMFDZVZY	AAFCG---XV		---FGHY	XQ-----ZY		MXPGNSAXYX
QAQEHGCAAZF	SPNAQXMZVF		ALW--PZF	FO-----TX		
-M---GVHDI	VAATAGAVF		VPHZNAY	TP---AAZAF		
YVVS---RNZY	XLH--ET		---GHGGY	VVATZNZZX		

Fig. 5 continued

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209290" hE02300T

Pooled Peptide  
Motif

P1 P2 P3 P4 P5 P6 P7 P8 P9  
T R P  
S E Q M Y K D H

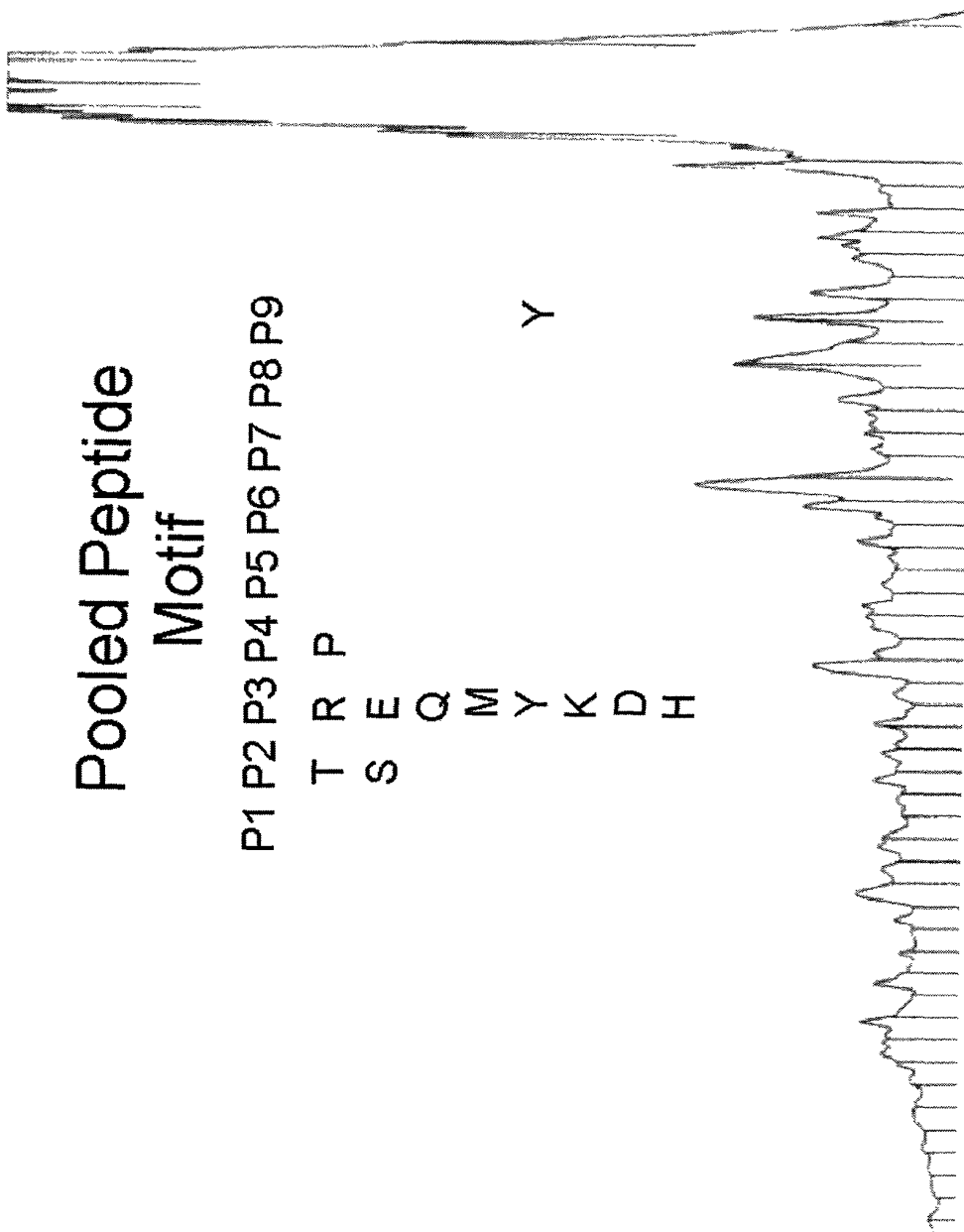


Fig. 6

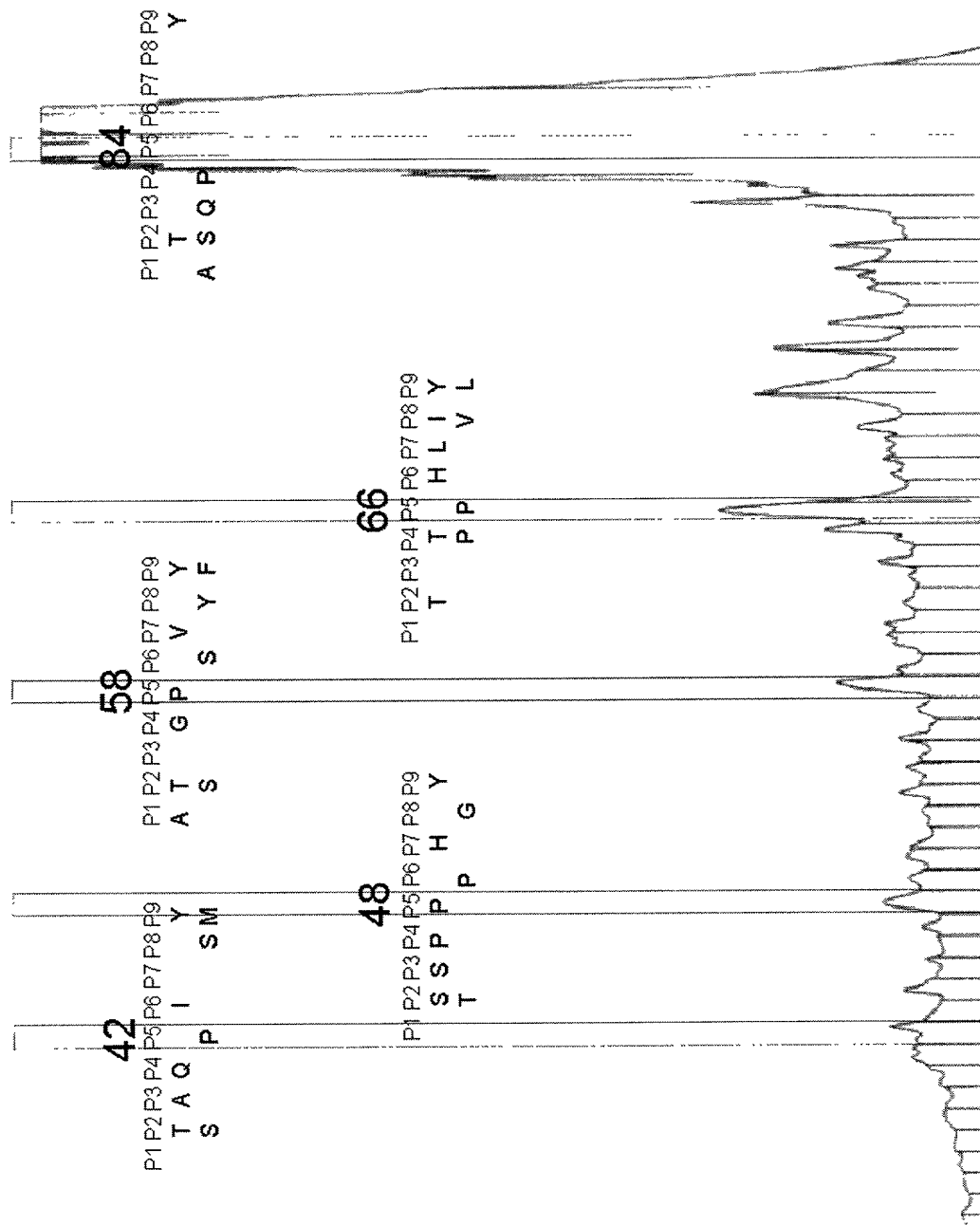


Fig. 7

Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen					
MNGRADFREP	NAEVPRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWERSYPL	
AATSMULTQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTQCE	
KFKKLENSPL	GEALRSGGAR	RSSPPGHYYQ	KSKYDSSVSG	QSSFVTSPAA	
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDNLEE	
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVKNKYG	
DTWDE					

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen					
MNGRADFREP	NAEVPRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWERSYPL	
AATSMULTQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTQCE	
KFKKLENSPL	GEALRSGGAR	RSSPPGHYYQ	KSKYDSSVSG	QSSFVTSPAA	
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDNLEE	
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVKNKYG	
DTWDE					

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

Fig. 8

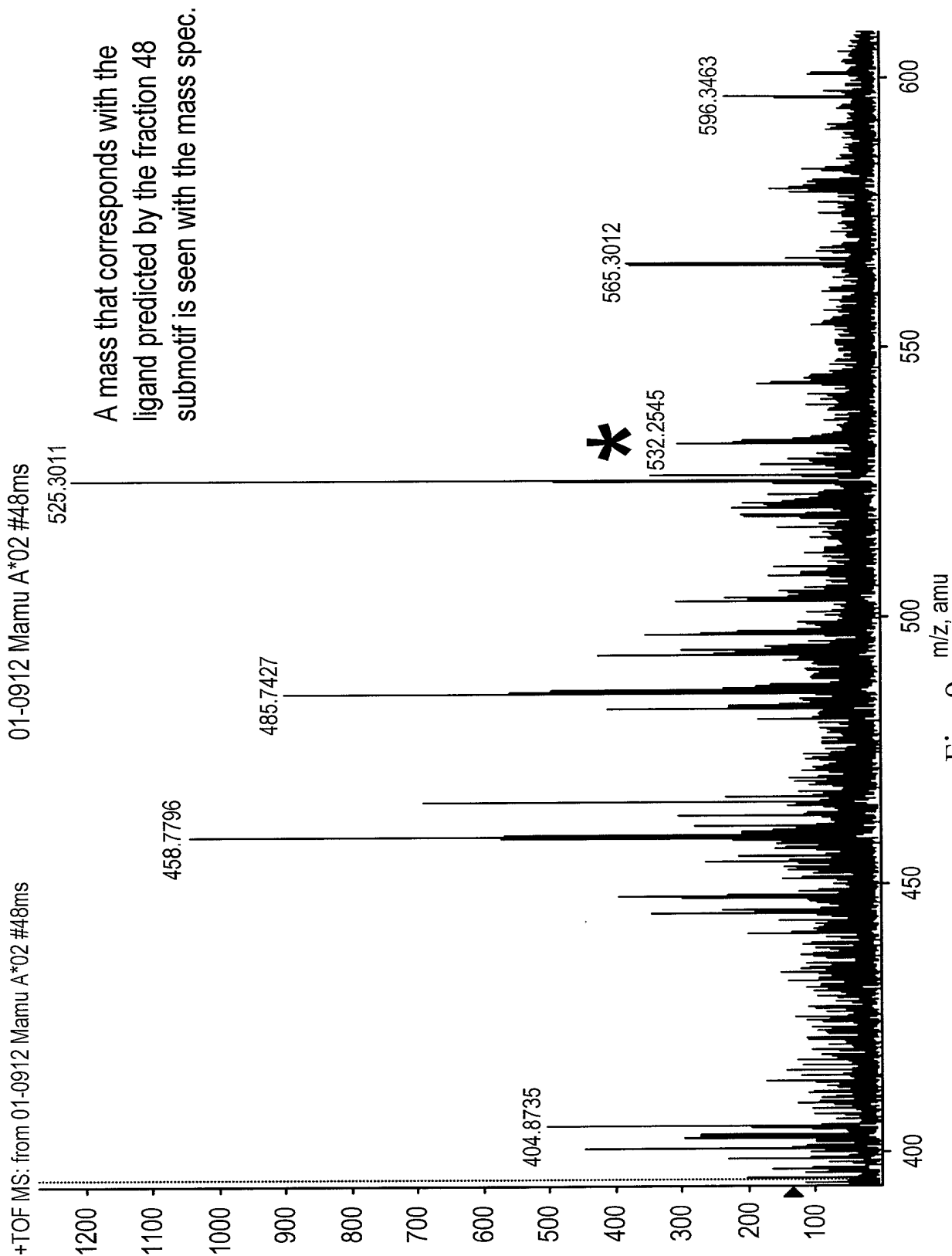
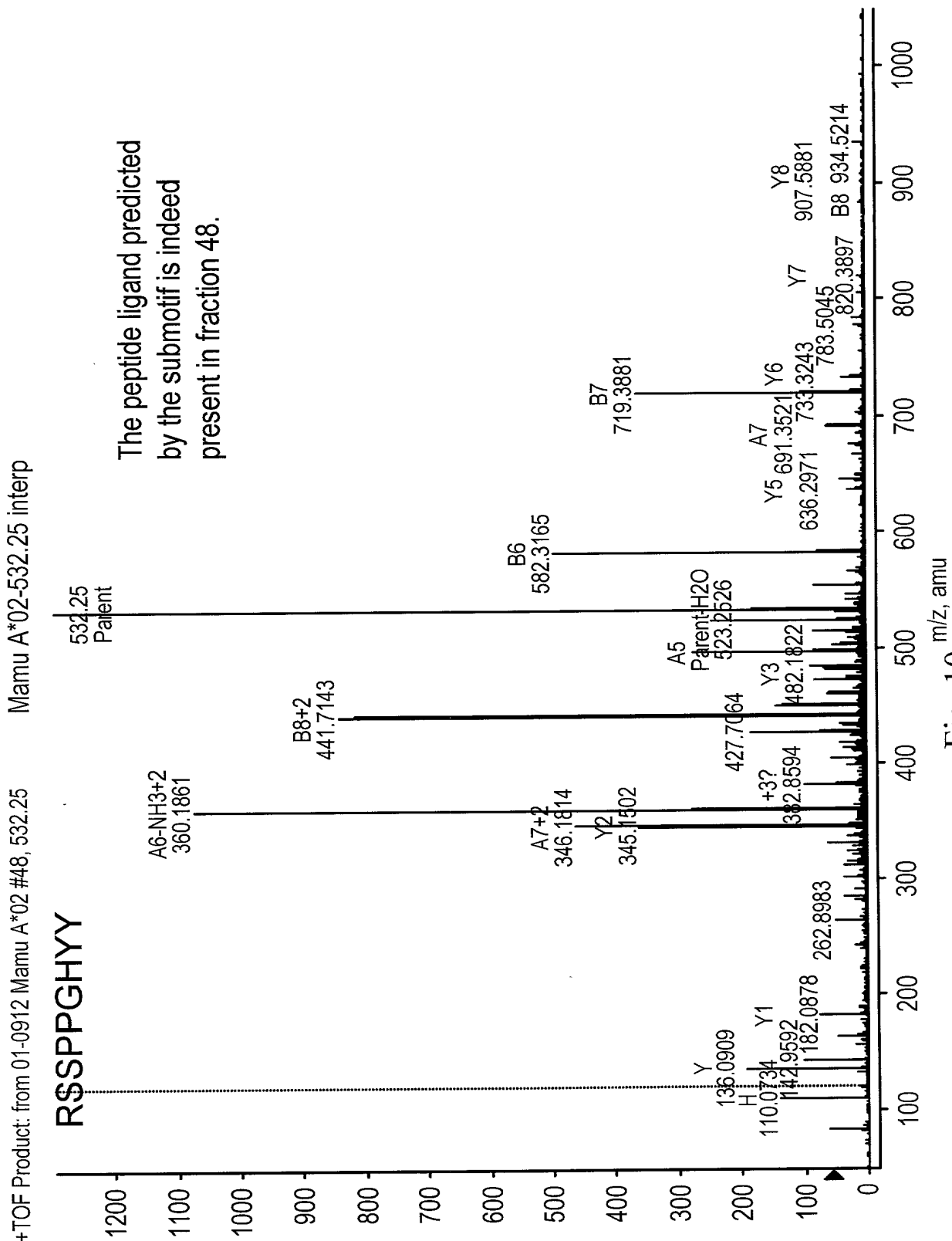


Fig. 9



Motif Data (Edman sequencing)

	1	%	fold	2	%	fold	3	%	fold	4	%	fold	5	%	fold	6	%	fold	7	%	fold	8	%	fold	9	%
<b>Dominant</b> 3.5 fold increases or more over prior round							F	9.20	11.18																	
							I	7.60	7.01																	
							N	6.20	4.11																	
							M	4.90	10.42																	
<b>Strong</b> 2.5-3.5 fold increase over prior round	K	31.50		R	53.80	2.57	Q	8.20	3.10	P	8.30	2.97													L	7.50
	R	15.50					K	5.40	2.67																	
	S	10.40					L	5.10	3.47																	
<b>Weak</b> 2.0-2.5 fold increase over prior round							A	5.80	2.08				M	4.90	2.28				T	7.70	2.12					
<b>Trace</b> 1.50-2.0 fold increase over prior round				Q	3.60	1.79	P	2.20	1.94	K	11.00	1.63	I	6.50	1.99							Q	11.40	1.93		
										S	6.00	1.68	F	4.20	1.83							N	5.70	1.55		
										V	5.30	1.99	H	1.80	1.67											

Fig. 11

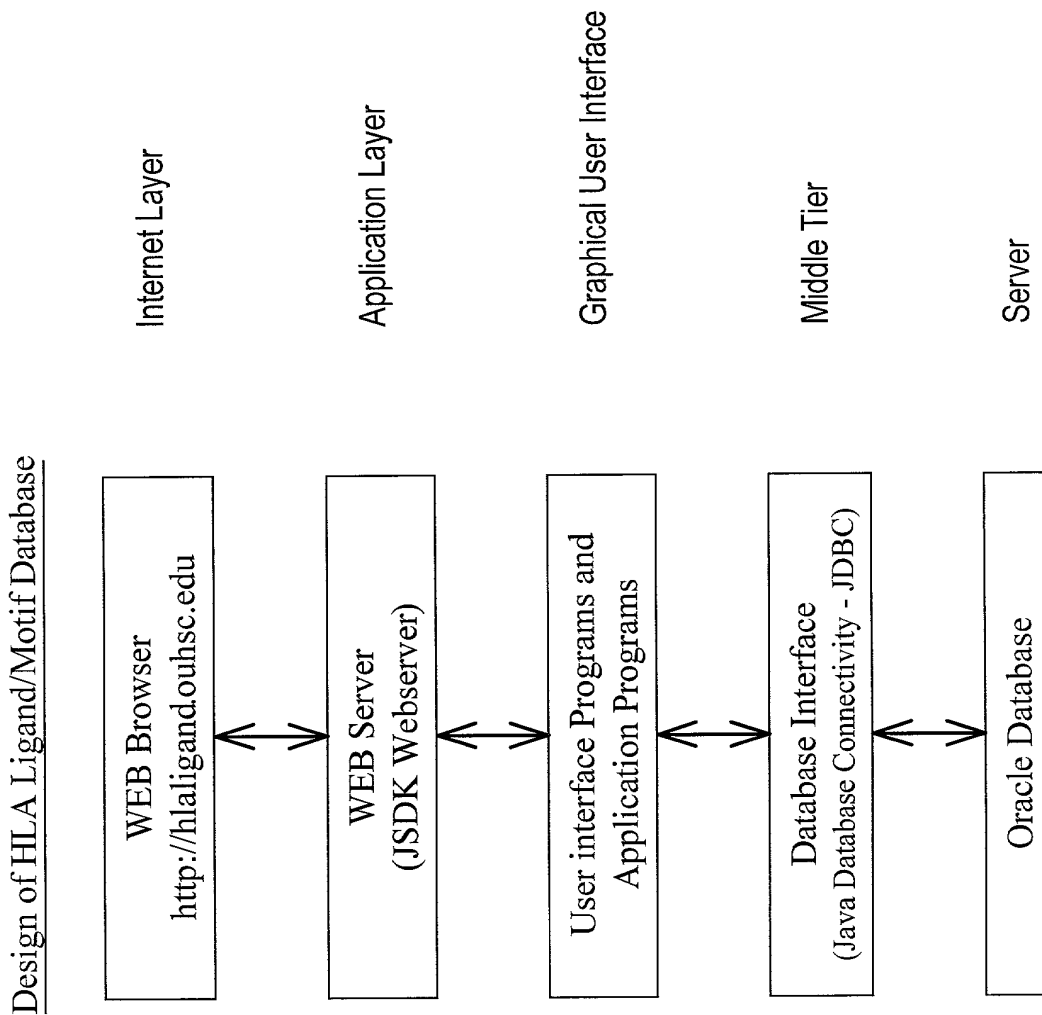


Fig. 12

Entity-Relationship (ER) Diagram for HLA Ligand/Motif Database

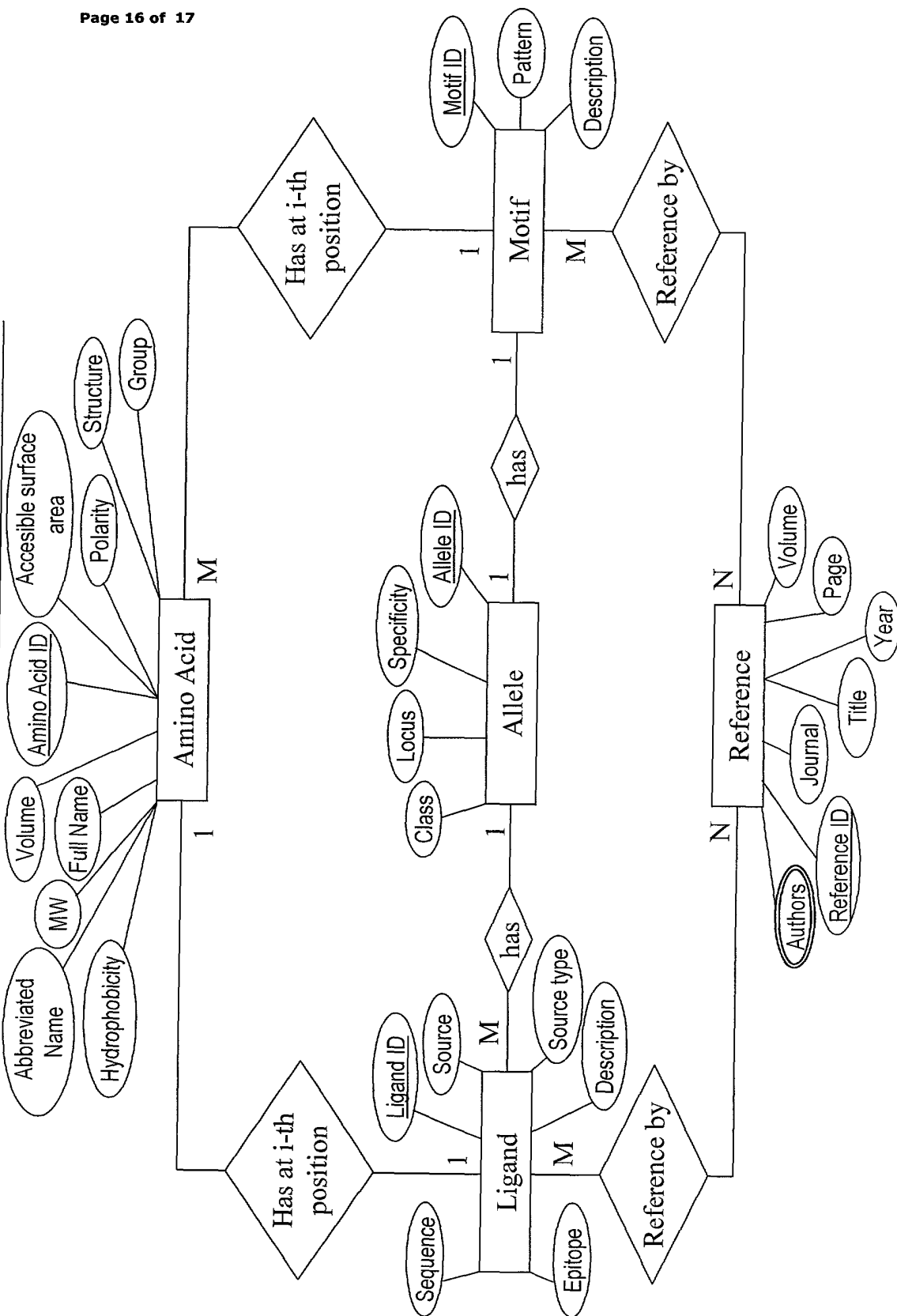


Fig. 13

UML Diagram for HLA Ligand/Motif Database

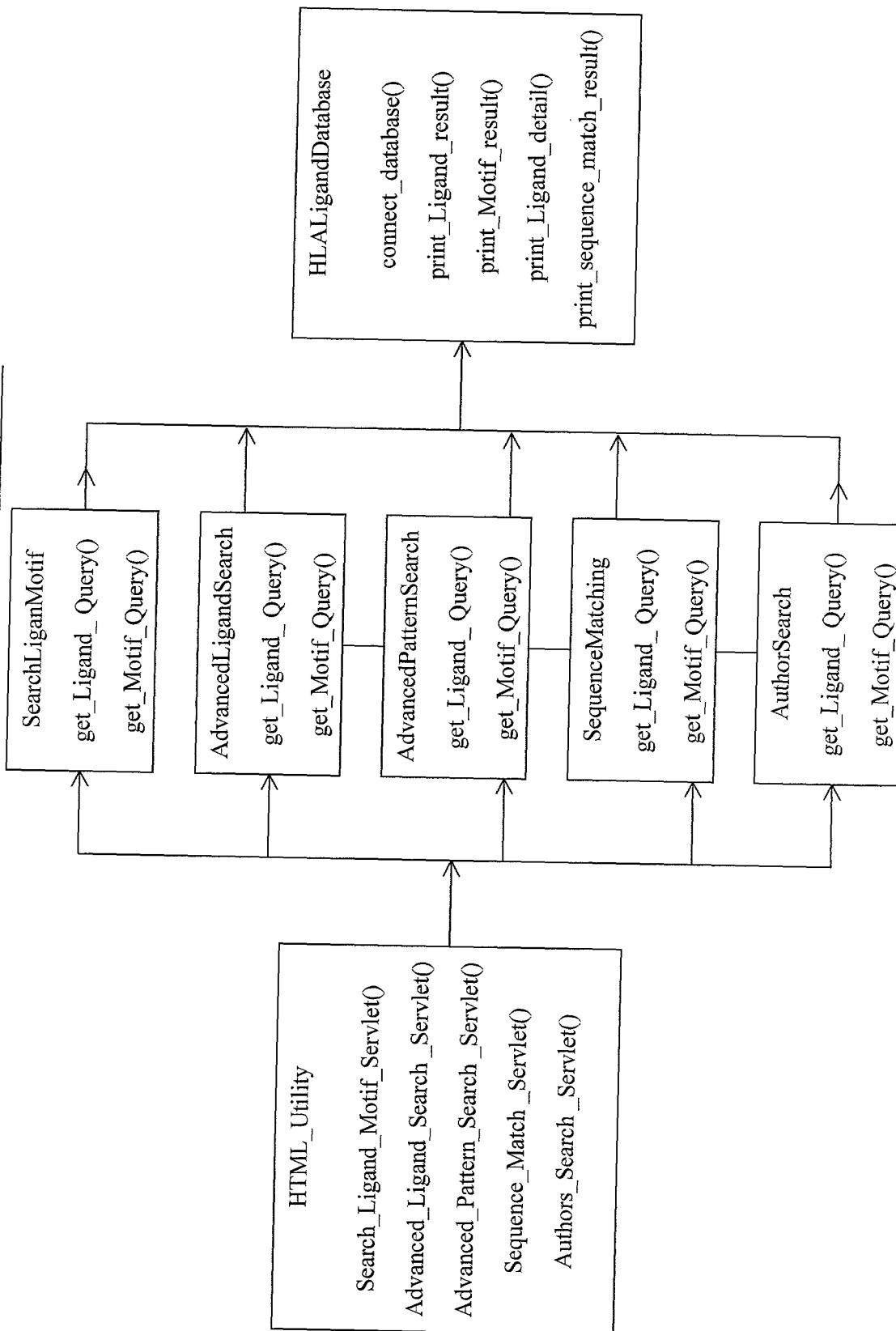


Fig. 14